The Role of DNA Damage and Extracellular Trap Formation in Atherosclerosis

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A thesis submitted in fulfilment of the requirements for the award of the degree Doctor of Philosophy (Science)

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Certificate of Original Authorship

I, Vickie Tang declare that this thesis, is submitted in fulfillment of the requirements for the award of PhD: Science, in the School of Maths and Physical Sciences at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This thesis contains materials that have been published in [1] and is shown in Chapter 3. This publication includes the results shown in Figures 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 3.10 and 3.11. I designed all the studies, analysed the data and wrote the drafts for the manuscript.

This document has not been submitted for qualifications at any other academic institutions.

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Vickie Tang

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“Nothing in life is to be feared, it is only to be understood.
Now is the time to understand more, so that we may fear less.”

- **Marie Curie**

“Even miracles take a little time.”

- **Fairy Godmother**

“You have brains in your head. You have feet in your shoes.
You can steer yourself any direction you choose.
You're on your own. And you know what you know.
And you are the one who'll decide where to go...”

- **Dr Seuss**

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Abstract

The primary cause of cardiovascular disease is atherosclerosis, which is an inflammatory disease resulting in plaque formation within blood vessels. Myeloperoxidase (MPO), an enzyme released by activated leukocytes in atherosclerosis catalyses the formation of hypochlorous acid (HOCl) which reacts readily with nucleic acids forming chlorinated nucleosides. There is increasing evidence that the formation of these chlorinated adducts has adverse effects, and elevated levels of these species are present in different inflammatory contexts, including atherosclerotic lesions.

The focus of Chapters 3 and 4 in this Thesis was to examine the effects of chlorinated nucleosides on endothelial cells (HCAEC) and smooth muscle cells (HCASMC) isolated from the human coronary artery. Studies in HCAEC and HCASMC showed that each of the chlorinated nucleosides studied incorporated into the RNA and DNA of these cells. However, only 8-chloroadenosine (8ClA) was shown to influence the metabolic activity and cell viability in HCAEC. 8ClA was converted to 8-ClATP, which was associated with alterations in the ability of HCAEC to perform glycolysis. Exposure of HCAEC to 8ClA also induced endoplasmic reticulum (ER) stress, resulting in apoptotic cell death and activation of the Nrf2 antioxidant stress response.

In Chapter 4, studies in HCASMC demonstrated that 8ClA and 5-chloro-2'-deoxycytidine (5CldC) decreased both the metabolic activity and proliferation of HCASMC. The effects of 8ClA on HCASMC were similar to those observed in HCAEC though less profound as the viability of HCASMC was unaffected, though some ER stress was apparent. In contrast, exposure of HCASMC to 5CldC resulted in alterations to pro-inflammatory

signalling, a large increase in the expression of MMP2 and MMP9 and phenotypic changes.

A potential source of chlorinated nucleosides under inflammatory conditions was explored in Chapter 5. The differentiation of PLB-985 cells into neutrophils was optimised, together with various treatment conditions to induce the productions neutrophil extracellular traps (NETs). The concentrations of modified nucleosides in both NET DNA and nuclear DNA of these cells was quantified using LC-MS/MS and it was shown that phorbol myristate acetate (PMA) and HOCl treatment gave 8-oxo-7,8-dihydro-deoxyguanosine (8oxodG) and 5CldC, respectively, in both NET and nuclear DNA.

Overall, the studies outlined in this Thesis provide insight into a potential new source of modified nucleosides in disease. These data show that chlorinated nucleosides are not only biomarkers of disease but can alter cellular function in both HCAEC and HCASMC. Therefore, these chlorinated adducts could play a role in the development and progression of atherosclerosis.

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Abbreviations

Abbreviation	Definition
18S	18S ribosomal RNA
2-NBDG	2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose
3-ClTyr	3-chlorotyrosine
5ClC	5-chlorocytidine
5CldC	5-chloro-2'-deoxycytidine
5CldU	5-chloro-2'-deoxyuridine
5ClU	5-chlorouridine
5ClUra	5-chlorouracil
8ClA	8-chloroadenosine
8-ClATP	8-chloro-ATP
8Cl-cAMP	8-chloro-cyclic AMP
8CldA	8-chloro-2'-deoxyadenosine
8CldG	8-chloro-2'-deoxyguanosine
8ClG	8-chloroguanosine
8oxodG	8-oxo-7,8-dihydro-deoxyguanosine
8oxoG	8-oxo-7,8-dihydroguanosine
Ad	Adenosine
AMP	Adenosine monophosphate
ANOVA	Analysis of variance
ApoB-100	Apolipoprotein B-100

$ApoE^{-/-}$	Apolipoprotein E knockout mice
APS	Ammonium persulfate
ATF4	Activating transcription factor 4
ATF6	Activating transcription factor 6
ATP	Adenosine triphosphate
ATRA	All-trans retinoic acid
BCA	Bicinchoninic acid
BSA	Bovine serum albumin
CCCP	Carbonyl cyanide m-chlorophenyl hydrazone
cDNA	Complimentary DNA
CHOP	CCAAT-enhancer-binding protein homologous protein
CTP	Cytidine triphosphate
CVD	Cardiovascular disease
CX43	Connexin 43
Cyt	Cytidine
dC	2'-deoxycytidine
dG	2'-deoxyguanosine
DMEM	Dulbecco's Modified Eagle Medium
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
ECAR	Extracellular acidification rate

ECD	Electrochemical detection
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
ER	Endoplasmic reticulum
ESI	Electrospray ionisation
ETC	Electron transport chain
FBS	Fetal bovine serum
FCCP	Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone
FITC	Fluorescein isothiocyanate
FPG	Formamidopyrimidine DNA glycosylase
GADD34	Growth arrest and DNA damage-inducible protein 34
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GCLC	Glutamate-cysteine ligase catalytic subunit
GC-MS	Gas chromatography mass spectrometry
GPx4	Glutathione peroxidase 4
GS	Glutathione synthetase
GTP	Guanosine triphosphate
HBSS	Hank's balanced salt solution
HCAEC	Human coronary artery endothelial cells
HCASMC	Human coronary artery smooth muscle cells
HDL	High-density lipoprotein
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HO-1	Heme oxygenase 1
HPLC	High performance liquid chromatography
ICAM-1	Intercellular adhesion molecule-1
IFNγ	Interferon γ
IL-1	Interleukin-1
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-13	Interleukin-13
IL-1β	Interleukin-1β
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-8	Interleukin-8
IRE1	Inositol-requiring enzyme 1
Keap-1	Kelch-like ECH-associated protein 1
LC-MS	Liquid chromatography mass spectrometry
LC-MS/MS	Triple quadrupole liquid chromatography mass spectrometry
LDL	Low density lipoprotein
LOD	Limit of detection
LPS	Lipopolysaccharide
M1	Pro-inflammatory macrophage
M2	Anti-inflammatory macrophage
MMP2	Matrix metalloproteinase 2
MMP9	Matrix metalloproteinase 9

Mnase	Micrococcal nuclease
MPO	Myeloperoxidase
MRM	Multiple reaction monitoring
mRNA	Messenger RNA
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NETs	Neutrophil extracellular traps
NMR	Nuclear magnetic resonance
NQO1	NADPH quinone dehydrogenase 1
Nrf2	Nuclear factor erythroid-2-related factor 2
OCR	Oxygen consumption rate
Olfml3	Olfactomedin Like 3
OPN	Osteopontin
oxLDL	Oxidised low-density lipoprotein
PAD4	Peptidylarginine deiminase 4
PBS	Phosphate buffered saline
PERK	PKR-like endoplasmic reticulum kinase
PI	Propidium iodide
PMA	Phorbol 12-myristate 13-acetate
PVDF	Polyvinylidene fluoride
qPCR	Quantitative real time polymerase chain reaction
RIPA	Radioimmunoprecipitation assay
RNA	Ribonucleic acid

ROS	Reactive oxygen species
RPL13	Ribosomal protein L13
RPMI	Roswell Park Memorial Institute medium
S100A4	S100 calcium binding protein A4
SDS	Sodium dodecyl sulphate
SEM	Standard error of the mean
SOD1	Superoxide dismutase 1
SOD2	Superoxide dismutase 2
SR-AI	Scavenger receptor-AI
SR-AII	Scavenger receptor-AII
sXBP1	Spliced X-box binding protein 1
TBST	Tris-buffered saline with 0.1% Tween 20
TEMED	Tetramethylethylenediamine
TFA	Trifluoroacetic acid
TLR	Toll-like receptor
TNFα	Tumour necrosis factor α
TRAIL	Tumour necrosis factor related apoptosis inducing ligand
Trx	Thioredoxin
TXNIP	Thioredoxin interacting protein
UDG	Uracil DNA glycosylase
UPR	Unfolded protein response
UTP	Uridine triphosphate
VCAM-1	Vascular cell adhesion molecule-1

Publications and conference presentations arising from this thesis

Publications

Tang, V., Macer-Wright, J., Fu, S., Rayner, B., Hawkins, C., *Chlorinated Nucleosides - A Novel Inducer of Endothelial Dysfunction in Atherosclerosis?* Free Radical Biology and Medicine, 2016. **100**: p. S152.

Tang, V., Macer-Wright, J., Fu, S., Rayner, B., Hawkins, C., *A role for chlorinated nucleosides in the promotion of inflammation and endothelial dysfunction in atherosclerosis?* Free Radical Biology and Medicine, 2017. **108**: p. S30.

Tang, V., Macer-Wright, J., Rayner, B., Fu, S., Hawkins, C., *The effect of chlorinated nucleosides on vascular cell function.* Free Radical Biology and Medicine, 2018. **120**: p. S92-S93.

Tang, V., Rayner, B., Fu, S., Hawkins, C., *Role of chlorinated nucleosides in the promotion of vascular smooth muscle cell dysfunction.* Free Radical Biology and Medicine, 2018. **128**: p. S103-S104.

Tang, V., Fu, S., Rayner, B., Hawkins, C., *8-Chloroadenosine induces apoptosis in human coronary artery endothelial cells through the activation of the unfolded protein response.* Redox Biology, 2019. **26**: p. 101274.

Poster presentations

Tang, V., Macer-Wright, J., Fu, S., Rayner, B., Hawkins, C.

"Chlorinated Nucleosides - A Novel Inducer of Endothelial Dysfunction in Atherosclerosis?"

23rd Annual Meeting of Society for Redox Biology and Medicine, 16th – 19th November 2016, San Francisco, USA.

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"A role for chlorinated nucleosides in the promotion of inflammation and endothelial dysfunction in atherosclerosis?"

OCC World Congress and Annual Society for Free Radical Research Europe, 21st – 23rd June 2017, Berlin, Germany.

Tang, V., Macer-Wright, J., Rayner, B., Fu, S., Hawkins, C.

"*The effect of chlorinated nucleosides on vascular cell function."*

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Tang, V., Rayner, B., Fu, S., Hawkins, C.

"*Role of chlorinated nucleosides in the promotion of vascular smooth muscle cell dysfunction."*

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Oral presentations

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"The role of chlorinated nucleosides in endothelial dysfunction and atherosclerosis."

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Tang, V., Rayner, B., Fu, S., Hawkins, C.

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